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Bionanotechnology for vaccine design

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There have been significant advances in the design of nanostructured scaffolds for eliciting robust immune responses. One method to produce strong immune responses is to emulate the appearance of a pathogen. Since pathogens such as viruses and bacteria often display multiple copies of ligands on their surfaces, the immune system is particularly sensitive towards multivalent displays of antigens. Consequently, when designing a vaccine, it is advantageous to decorate a nanostructured surface with multiple copies of an antigen. This review highlights the design and efficacy of a diverse set of recently developed nanostructured vaccine scaffolds.

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Introduction

Vaccines constitute one of the most effective and cost-effective tools to prevent disease [1]. Since many of the interactions of the immune system with pathogens are multivalent, approaches for displaying multiple copies of antigens from scaffolds have been of great interest for vaccine design. This review focuses on recent advances in the development of nanostructured scaffolds for vaccine design (Figure 1), with special emphasis on reports published within the last 3 years.

Virus-like particles (VLPs)

VLPs mimic the morphology of a virus particle, but usually lack genetic material, and cannot replicate, mutate, or recombine [2••,3]. They display a dense array of antigens on their surface and can therefore induce strong immune responses [2••,3]. VLP-based vaccines against hepatitis B, human papillomavirus, and hepatitis E are among the most effective human vaccines, with efficacies ranging from 95 to 100% [4••,5]. As a result, there is a continuing interest in designing better vaccines based on VLPs.

Marsian *et al.* [2••] reported the production of synthetically stabilized poliovirus (PV) VLPs in plants. They expressed VLPs of the stabilized PV3 mutant SktSC8 (sVLPs). Structure determination by cryo-electron microscopy (cryo-EM) revealed that the sVLPs adopted an antigenic conformation similar to that of wt PVs (Figure 2). Immunizing transgenic mice with the plant-expressed sVLPs induced similar neutralizing antibody responses to inactivated PV vaccines (IPV) and protected animals from a challenge with virulent virus at levels similar to those induced by IPV (Figure 2) [2••]. The sVLPs may therefore represent an alternative to IPVs for the prevention of polio.

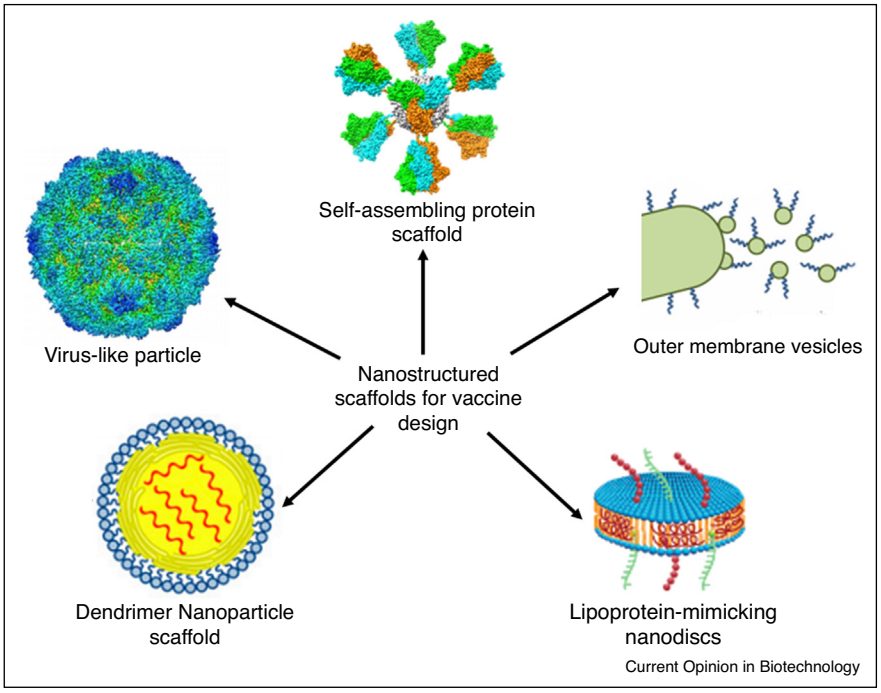
Zeltins *et al.* [6] explored the VLP-based design of 'therapeutical vaccines' — those aimed at blocking endogenous molecular pathways in order to alter the course of already established non-infectious conditions. They engineered VLPs derived from Cucumber Mosaic Virus (CMV) to incorporate an internally fused T-cell stimulatory epitope derived from Tetanus toxoid. The resulting VLPs (termed CMV_{TT}) elicited robust antibody responses even in old mice, and employing low vaccine doses, against several antigens including interleukin-17 and β-amyloid.

Moura *et al.* [7] designed Qβ VLPs presenting approximately 540 copies of the α-Gal trisaccharide (Qβ-α-Gal). Vaccination with Qβ-α-Gal nanoparticles protected α-Gal-T knockout mice against *Leishmania* challenge. In other intriguing work, Wei *et al.* [8•] designed self-amplifying VLPs (AVLPs) that can deliver and amplify foreign genes in target cells without producing progeny. They generated AVLPs that express the influenza antigen hemagglutinin (AVLP-H5) as well as those expressing both hemagglutinin and the M1 matrix protein (AVLP-M1H5) [8•]. Vaccination with a single dose of AVLP-H5 or AVLP-M1H5 completely protected mice against a lethal challenge with an H5N1 influenza virus strain [8•].

Self-assembled protein and peptide scaffolds

Just as the self-assembly of viral structural proteins can generate VLPs, the assembly of other proteins and peptides can be used to create nanostructured scaffolds for antigen display and immunization. Kanekiyo *et al.* [9] previously fused the ectodomain of the influenza hemagglutinin (HA) protein to ferritin — a protein that naturally forms nanoparticles composed of 24 identical units.

Figure 1



Different types of nanostructured scaffolds for vaccine design. The scaffolds discussed in this review include virus-like particles (Adapted from Ref. [2••], licensed under CC BY 4.0. https://www.nature.com/articles/s41467-017-00090-w), self-assembling protein scaffolds (Adapted from Ref. [4••], licensed under CC BY 4.0. https://www.nature.com/articles/ncomms12041), outer membrane vesicles (Adapted from Ref. [26••], licensed under CC BY 4.0. https://www.nature.com/articles/srep24931), lipoprotein-mimicking nanodiscs (Adapted by permission from Macmillan Publishers Ltd.: *Nature Materials*, Ref [28••;], copyright 2017. https://www.nature.com/nmat/), and dendrimer nanoparticle scaffolds (Adapted from Ref. [35••]).

Assembly of the HA–ferritin fusion protein generated eight trimeric HA spikes on the nanoparticle surface. A vaccine based on this nanoparticle also elicited neutralizing antibodies targeting the HA stem — a highly conserved HA domain.

More recently, Yassine *et al.* [10••] used a similar approach to display an HA stabilized stem-only immunogen (H1-SS, Figure 3a). Characterization of the self-assembled nanoparticles by cryo-electron microscopy once again revealed symmetrical, spherical particles, each with eight spikes protruding from the surface. The nanoparticles were recognized by several stem-directed antibodies in ELISA and biolayer interferometry measurements, confirming that the HA stem structure was preserved in the assembled nanoparticles [10••]. Vaccination of mice and ferrets with the nanoparticles elicited broadly cross-reactive antibodies that completely protected mice (Figure 3b) and partially protected ferrets against a lethal heterosubtypic H5N1 influenza virus challenge [10••].

Self-assembled ferritin-based nanoparticles have also been used to display trimeric HIV-1 antigens [4••,11]. The BG505.SOSIP.664 gp140 trimer — derived from HIV-1 strain BG505 with a number of stabilizing mutations — is an excellent antigenic and structural mimic of the native viral spike [4••,12]. He *et al.* [4••] designed ferritin-based nanoparticles displaying various forms of the stabilized gp140 trimer (Figure 3c). The nanoparticles bound to broadly neutralizing antibodies with sub-picomolar affinities (Figure 3d) and also demonstrated a significantly more robust triggering of B cells than individual gp140 trimers. The authors also showed that tuning the spacing between antigens on the nanoparticle surface was important for efficient nanoparticle assembly as well as antibody access to epitopes near the nanoparticle surface [4••].

McComb *et al.* [13] developed an epitope-targeted anthrax vaccine by presenting peptides from the *Bacillus anthracis* protective antigen on Tobacco Mosaic Virus (TMV). The TMV capsid is formed by the assembly of 2130 coat protein monomers [13]. McComb *et al.* [13] successfully expressed two peptide epitopes on TMV and showed that antibodies induced by these vaccine constructs cross-reacted with native anthrax toxin. Partial toxin neutralization was also observed *in vivo*, demonstrating the feasibility of this approach for vaccine design.

Kim *et al.* [14] constructed a polymeric IgG scaffold (PIGS) that incorporates multiple copies of the consensus domain III sequence of the dengue glycoprotein E